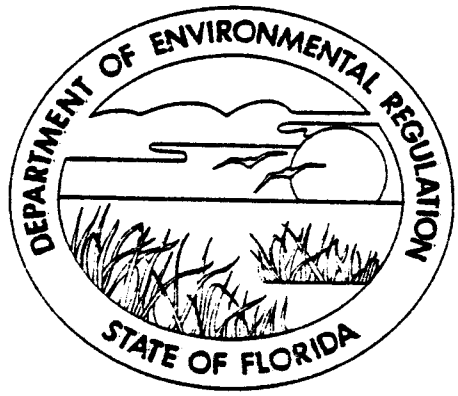


BIOASSAYS OF THE BUCKMAN STREET SEWAGE TREATMENT PLANT JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0026000

SAMPLED 8/2/83

Biology Section
Bureau of Water Analysis
September 20, 1983

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EXECUTIVE SUMMARY

Buckman St. STP, Jacksonville, NPDES #FL0026000, on 3 to 5 August, 1983.

The pre-chlorinated effluent from this municipal facility, which treats a considerable amount of industrial wastes, was acutely toxic to Mysidopsis bahia, producing a 48 hour LC_{50} of 99.3%. The concentrations of total and unionized ammonia detected in this sample (7.7 mg/l and 0.133 mg/l, respectively) may have contributed to the toxicity, but were insufficient to have been the sole cause of it. The chlorinated effluent, in which a total residual chlorine concentration as high as 1.95 mg/l was detected, was extremely toxic to M. bahia, producing a 48 hour LC_{50} of 14.3%. Most of this toxicity was probably due to the total residual chlorine. The de-chlorinated effluent was not acutely toxic to M. bahia, only 3 of 10 shrimp died in the 100% effluent concentration, insufficient to calculate an LC_{50}.

Chemical analyses of these effluents indicated the presence of several unidentified organic compounds, of unknown concentrations, which may or may not have, contributed to the observed toxicity.

INTRODUCTION

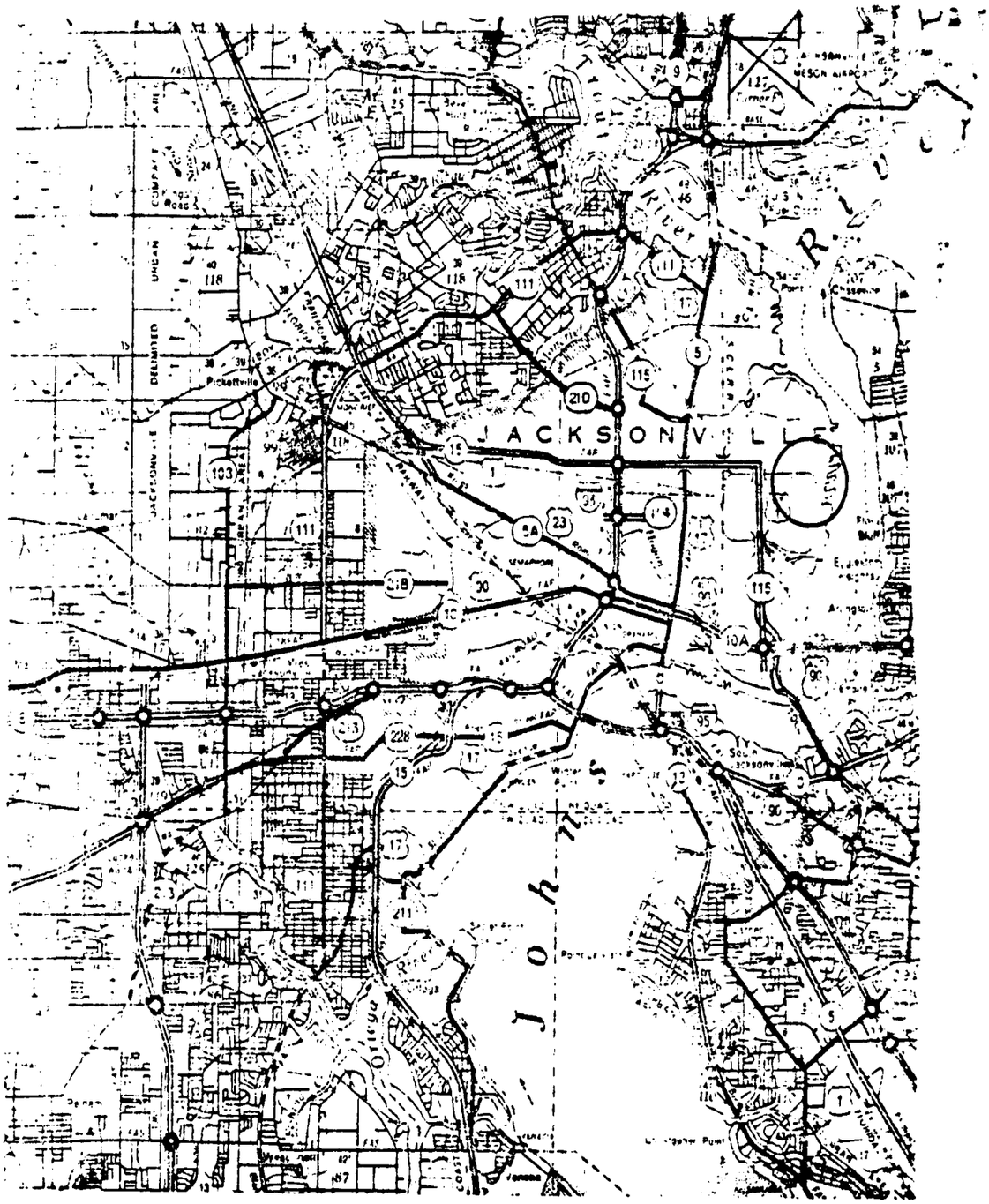
The Buckman Sewage Treatment Plant is located at 2221 Buckman St., in the center of Jacksonville, Duval County, Florida (Fig. 1). This 35 MGD capacity activated sludge treatment plant serves a population of approximately 200,000, and provides secondary treatment for wastes from a variety of major and minor industrial sources. Treatment consists of primary sedimentation, sludge aeration, secondary clarification and chlorination. Discharge is to the St. John's River, 500 feet from shore. Recent NPDES Compliance Inspection Reports have indicated that this facility has been discharging in excess of its design capacity, and also that, the discharge contains only partially treated wastes.

In October, 1979, the Biology Section performed a static acute toxicity bioassay on pre-chlorinated effluent collected from the secondary clarifier, to determine the toxicity of effluent from this facility. Results indicated a 48 hour LC_{50} of 50%, based on the test organism, Mysidopsis bahia (FDER, 1979). Because of the complex nature of the effluent, toxicity could not be attributed to a single component.

In August, 1981, additional bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this facility, to further evaluate their toxicity. The results of those tests indicated that the pre-chlorinated and chlorinated effluents were acutely toxic to Mysidopsis bahia (approximate LC_{50}'s were 64% and 52%, respectively), while the de-chlorinated effluent was lethal to a number of shrimp (FDER 1981).

In June and July, 1982, five additional bioassays were performed on pre-chlorinated, chlorinated, and de-chlorinated effluents from the Buckman St. facility. Those tests were performed using freshwater test organisms, since the salinity of the normally saline St. Johns River at that time had decreased to approximately 1 ppt. The effluents were variously lethal to the test organisms, most notable were the 48 hour LC_{50}'s of 6.3% for Daphnia pulex (chlorinated effluent), and 77.5% for Simocephalus serrulatus (de-chlorinated effluent). The toxicity at that time was attributed to total residual chlorine and to the presence of a variety of chemical constituents (FDER 1982).

Since effluent from the Buckman St. STP has been shown to exhibit a continued toxicity problem, additional bioassays were recently performed, on 3 to 5 August, 1983, on samples of pre-chlorinated, chlorinated and de-chlorinated effluents. These latest tests were performed with the saltwater test organism, Mysidopsis bahia, since the salinity of the St. Johns River at the time of sampling was moderately saline. The results of these latest tests are presented in this report. Those involved in performing these tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.



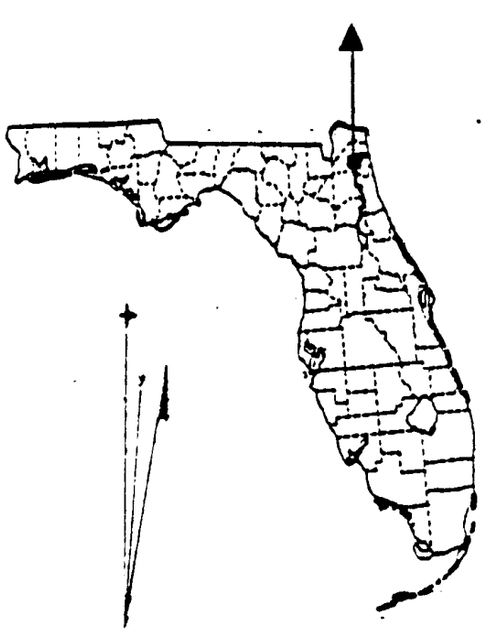


Figure 1. Portions of Jacksonville, Duval County, Florida, including location of the Buckman St. STP.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organism selected for this study is among those recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). The life history and maintenance procedures used to culture the organism are detailed below.

History and Maintenance of Bioassay Organisms

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 2°C. Adult or large juvenile mysids were used in this test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Three separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Buckman St. STP. The tests were performed from 1100 hours on 3 August to 1100 hours on 5 August, 1983. The samples were collected by Ms. Teresa Rhodes of DER's Jacksonville office at 1409 hours on 2 August, 1983, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biology Section adopted the following test procedure:

Test #1. Pre-chlorinated Effluent (Table 2). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination, and was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 3). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent (Table 4). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate. This test was intended to evaluate the combined toxicity of the effluent and the chlorine reaction products.

Saltwater for dilution of the effluent samples was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 25.0 ppt). This saltwater diluent was then adjusted to a moderate salinity (18.0 ppt) characteristic of the receiving body of water (St. Johns River) by adding an appropriate amount of DER well water. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the effluent, achieving a range of final salinities (8.5 ppt to 17.5 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerance). Four control groups (Table 5) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included:

1) Diluent Control - Turkey Point water (25.0 ppt), adjusted with well water to a salinity of 18.0 ppt. This represents the highest concentrations of both Turkey Point water and well water used in the bioassays, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity

of the highest concentration of artificial sea salts used in the bioassays, as well as for tolerance of the organisms to the lowest salinity used in the bioassays.

3) pH Adjusted Effluent Control - Pre-chlorinated effluent (100%) which has been adjusted to a salinity of approximately 7 ppt using Ocean 50®, and which has then been pH adjusted using hydrochloric acid to the original pH of the effluent prior to salinity adjustment. This test ensures that toxicity observed in the bioassay is not merely due to a change in pH caused by the sea salts.

4) Acid Control - Well water, adjusted with Ocean 50® to a salinity of approximately 7 ppt, with an amount of hydrochloric acid added equal to that added in the pH Adjusted Effluent Control. This tests for toxicity in the pH Adjusted Effluent Control due to the hydrochloric acid.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each randomly arranged test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt and 20 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. The salinity (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the Sea Salts control and the 100% effluent test concentrations at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2 through 5.

Table 1. Concentration Series and Volumes Used for These Static Bioassays

Mydidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	------	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Buckman St. STP, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 3 to 5 August, 1983.

Table #1 Pre-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/N (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	8.3	6.9	7.2	8.1	8.0	7.8	20.6	19.3	18.9				
5.6%	5	5	5	8.3	6.9	7.2	8.1	8.0	7.8						17.5	15.9
10.0%	5	5	5	8.2	7.0	7.0	8.1	7.9	7.8	20.4	19.3	18.9				
10.0%	5	5	5	8.2	7.0	7.0	8.1	7.9	7.8						16.8	15.3
18.0%	5	5	5	8.0	7.0	6.9	8.0	7.9	7.8	20.3	19.4	19.0				
18.0%	5	5	5	8.0	6.8	6.9	8.0	7.9	7.8						15.2	14.5
32.0%	5	5	5	7.9	7.0	6.8	7.9	8.0	7.9	20.1	19.5	19.0				
32.0%	5	5	4	7.9	7.1	6.8	7.9	7.9	7.8						13.0	12.5
56.0%	5	5	5	7.1	6.8	6.8	7.7	8.0	7.9	19.9	19.4	18.9				
56.0%	5	5	5	7.1	6.9	7.0	7.7	7.9	8.0						8.5	8.4
100.0%	5	5	2	6.1	7.0	7.5	8.1	8.2	8.1	19.8	19.4	18.9	0.42/7.7	--		
100.0%	5	5	3	6.1	6.9	7.5	8.1	8.2	8.2						7.5	7.4
pH adj. 100% eff.[b]	5	5	2	7.3	7.5	7.2	7.6	8.0	8.0	19.5	19.4	18.9	0.13/7.7	--		
pH adj. 100% eff.[b]	5	5	2	7.3	7.5	7.2	7.6	8.0	8.1						7.2	7.5

Remarks: [a] Total Residual Chlorine: None Detected (HACH DPD Method).

[b] The data for the pH adjusted 100% effluent control are presented here, as well as in Table 5, since the mortality data from both 100% effluents were combined to calculate an LC_{50} for the pre-chlorinated effluent.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Buckman St. STP, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 3 to 5 August, 1983.

Test #2 Chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	8.2	7.3	7.0	8.2	8.0	7.8	20.6	19.1	18.8				
5.6%	5	5	5	8.2	7.3	7.0	8.2	8.0	7.8						17.2	16.5
10.0%	5	5	5	8.3	7.4	7.2	8.1	8.0	7.9	20.4	19.1	18.8				
10.0%	5	5	5	8.3	7.5	7.3	8.1	8.0	7.9						16.6	16.0
18.0%	5	2	0	8.5	7.6	7.6	8.0	8.1	8.0	20.3	19.0	18.8				
18.0%	5	2	1	8.5	7.6	7.6	8.0	8.0	8.0						15.2	14.8
32.0%	5	0	0	8.3	7.8	--[b]	7.9	8.1	--[b]	20.2	19.0	--[b]				
32.0%	5	0	0	8.3	7.9	--	7.9	8.1	--						12.9	--[b]
56.0%	5	0	0	8.3	8.0	--	7.7	8.1	--	20.1	19.0	--				
56.0%	5	0	0	8.3	8.0	--	7.7	8.1	--						8.5	--
100.0%	5	0	0	8.3	8.2	--	8.2	8.3	--	20.1	19.2	--	0.54/7.8	--		
100.0%	5	0	0	8.3	8.2	--	8.2	8.3	--						7.2	--

Remarks: [a] Total Residual Chlorine: 1.5 mg/l (HACH DPD Method), 1.95 mg/l (Iodometric Method I).

[b] Remaining parameters were not recorded since all organisms were dead by hour 24 of the test.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Buckman St. STP, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 3 to 5 August, 1983.

Test #3 De-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/N (mg/ℓ)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	8.4	7.1	7.4	8.2	8.0	7.8	20.3	19.1	18.7				
5.6%	5	5	5	8.4	7.2	7.4	8.2	8.0	7.8						17.1	16.4
10.0%	5	5	5	8.4	7.0	7.2	8.1	8.0	7.8	20.2	19.3	18.8				
10.0%	5	5	5	8.4	6.9	7.2	8.1	7.9	7.8						16.8	16.0
18.0%	5	5	5	8.5	6.9	7.0	8.1	8.0	7.8	20.1	19.1	18.8				
18.0%	5	5	5	8.5	7.0	7.0	8.1	8.0	7.8						15.1	14.7
32.0%	5	5	5	8.5	7.1	6.7	8.0	7.9	7.8	20.1	19.1	18.8				
32.0%	5	5	5	8.5	7.1	6.8	8.0	7.9	7.8						13.8	12.3
56.0%	5	5	5	8.5	7.2	7.1	7.8	8.0	7.9	20.1	19.0	18.7				
56.0%	6[b]	6	6	8.5	7.2	7.3	7.8	7.9	7.9						8.8	8.5
100.0%	5	5	3	8.3	7.3	7.4	8.2	8.2	8.2	20.5	19.1	18.8	0.58/8.2	--		
100.0%	5	5	4	8.3	7.2	7.5	8.2	8.2	8.2						7.0	7.0

Remarks: [a] Total Residual Chlorine: None Detected (HACH DPD Method)

[b] Extra organism loaded by mistake, allowed to remain.

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Buckman St. STP, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 3 to 5 August, 1983.

Control Series

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Diluent Control	5	5	5	8.5	7.0	7.3	8.2	8.0	7.8	20.3	19.3	19.0				
Diluent Control	5	5	5	8.5	7.0	7.3	8.2	8.0	7.8						18.0	17.0
Sea Salts Control	5	5	5	8.8	7.5	7.8	8.8	8.4	8.1	20.6	19.3	18.9	0/0	--		
Sea Salts Control	5	5	5	8.8	7.5	7.8	8.8	8.5	8.2						7.0	6.9
pH Adj. Effluent[a]	5	5	2	7.3	7.5	7.2	7.6	8.0	8.0	19.5	19.4	18.9	0.13/7.7	--		
pH Adj. Effluent[a]	5	5	2	7.3	7.5	7.2	7.6	8.0	8.1						7.2	7.5
Acid Control	5	4[b]	4	8.8	7.5	7.8	7.6	8.0	7.9	21.5	19.3	18.9				
Acid Control	5	5	5	8.8	7.6	7.8	7.6	7.9	7.9						7.0	6.9

Remarks: [a] 100% pre-chlorinated effluent used for this control.

[b] One organism jumped out between 0 and 24 hours.

Table 6. LC_{50} Data for Bioassays of the Buckman St. STP, NPDES #FL0026000, on 3 to 5 August, 1983.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: M. bahia Pre-chlorinated Sample	Binomial	No Mortality	96.0% (56 and ∞), b
	Moving Average		a
	Probit		99.3% (76 and 196), b, c
Test #2: M. bahia Chlorinated Sample	Binomial	16.7% (10 and 32)	14.3% (10 and 18)
	Moving Average	a	a
	Probit	a	a
Test #3: M. bahia De-chlorinated Sample	Binomial	No Mortality	a
	Moving Average		a
	Probit		a

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

[b] Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

[c] This LC_{50} value is reported in the text since it is the most statistically conservative value of the data set.

Table 7. Results of Chemical Analyses Performed on Effluents from the Buckman St. STP, NPDES #FL0026000, collected 2 August, 1983.

Organics

	Pre-chlorinated Effluent	Chlorinated Effluent
Acid Extractables	N.D.	N.D.
Base Neutral Extractables	One unidentified compound	N.D.
Pesticide Extractables	Four unidentified compounds	Four unidentified compounds

Metals

Arsenic	N.D.
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	N.D.
Lead	N.D.
Mercury	< 0.2 µg/l[a]
Nickel	N.D.
Selenium	N.D.
Silver	N.D.

N.D. = None Detected

[a] Actual value is known to be less than value given.

RESULTS AND DISCUSSION

The three effluents from the Buckman St. facility all exhibited some degree of toxicity to the test organism, Mysidopsis bahia. The pre-chlorinated effluent was acutely toxic to the shrimp, producing a 48 hour LC_{50} of 99.3% (Table 6). The chlorinated effluent was also acutely toxic, producing a 48 hour LC_{50} value of 14.3% (Table 6), due primarily to the total residual chlorine (1.95 mg/l, Table 3) present in that sample. The de-chlorinated effluent was not acutely toxic to M. bahia, but did produce some lethality (3 dead of 10 shrimp in the 100% effluent concentration, Table 4). The 96 hour LC_{50}'s for these effluents would be less than or equal to their 48 hour values. The moderate amounts of total and unionized ammonia detected in these effluents (7.7 mg/l to 8.2 mg/l and 0.13 mg/l to 0.58 mg/l, respectively) may have contributed to the toxicity, but could not have been the sole cause of it.

The DER Chemistry Section has analyzed samples of effluent from the Buckman St. facility for certain organic compounds and metals (Table 7). These analyses indicated the presence of several unidentified organic compounds, of unknown concentrations, which may, or may not have, contributed to the observed toxicity. Of the metals tested for, none were found in measurable amounts in the final effluent from the Buckman St. facility.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as one-twentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one-half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existance of a mixing zone, whether as a specific part of the permit or based on the existance of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters (17-3.061) are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic (17-3.061(2)(p)); that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters (17-3.051(1)(d)) applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 (17-4.244(4)). At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test

procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

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